FURTHER NOTES ON THE REGENERATION OF THE FINS OF FUNDULUS HETEROCLITUS.

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During the last summer the writer while engaged as assistant at the biological laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass., conducted various experiments on *Fundulus heteroclitus.* Since a number of these can be conveniently grouped under the heading *regeneration* they are collected and presented in the present paper. Various factors determining regeneration have been under discussion and it was thought that these should be tested using *Fundulus h.* as a type since it is a representative teleost and that therefore results might differ from those obtained in crustaceans, for example, and that certain principles deduced from crustacean work might possibly have to be modified. Thanks are due to Dr. F. B. Sumner as director of the laboratory of the Bureau of Fisheries for assistance in providing the necessary apparatus and other facilities.

The following experiments were designed to show: (*a*) the relation of temperature to regeneration, (*b*) the relation of degree of injury to regeneration, (*c*) the relation of length and weight (*i. e.*, age) of specimen to regeneration, (*d*) the relation of amount of food to regeneration, (*e*) adaptation in regeneration.

(A) Relation of Temperature to Regeneration.

With a view of testing this the caudal fin of ten *Fundulus h.* were cut in a line as near as possible to the origin of the caudal fin rays. The same procedure was followed in all the later cases to be described. These fish were placed in a chest or jar with three liters of sea water and the jar placed in an ice chest where the temperature averaged 14°C. The water was changed every day or so. On August 24 five fish had survived. The fish had not been fed. The survivors had been under observations for 41 days or almost six weeks. But in those survivors there was no sign of regeneration. With fishes in normal conditions regenera-

tion is evident after the first few days after operation. In order to further test this point and ascertain whether lack of food had anything to do with the result, two lots of fishes were operated on in a similar manner on September 1. Each lot was placed in a jar in three liters of sea water and both jars placed in the ice chest. One lot was fed and the other not fed. On September 14 nine tenths of those that had not been fed had survived and seven tenths of the others. There was no sign of regeneration in either lot. It might be added that in case of another experiment set up at the same time that regeneration was evident a few days after. (See Table III.)

It may be of interest to note that two of the survivors mentioned in the first experiment were placed in a shallow jar surrounded by running sea water, and in two weeks time the caudal fin showed a regeneration of 2 mm. indicating that just as soon as the temperature was raised to the normal regeneration began. It should also be noted that the temperature necessary to inhibit regeneration was not an extremely low one, and that this indicates that the power of regeneration is sensitive to lowering of temperature.

(B) Relation of Amount of Injury to Regeneration.

In 1902 Zeleny observed that when two chelæ of *Gelasimus*, the fiddler crab, were removed each of the regenerating buds grows more rapidly than does the single one when only one chela is removed. In 1903 the same author found that the regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the number of arms removed, *i. e.*, that animals with the greater number of arms removed regenerate more rapidly. The greater the degree of injury the more rapid the regeneration. In 1905 Zeleny in an experiment extending over 181 days using the common cray-fish, *Cambarus propinquus*, found that in the series with the greater degree of injury (Series B) each chela regenerates more rapidly than the single chela of those with the lesser degree of injury (Series A). in Series A consisting of 36 individuals the right chela was removed at its breaking joint. In series B consisting of 41 individuals the two chelæ and the last two pairs of walking legs were similarly removed. It is to be

regretted that the same author did not include a third series in which the injury was midway between that in A and B to see whether regeneration in that third series would be intermediate between that of series A and B. Thus Zeleny shows that in the cray-fish the greater degree of injury has a stimulating effect on regeneration.

On the other hand Emmel ('04) removed the right cheliped in the lobster immediately after moulting and allowed it to regenerate for the period between moults then removed it again after that moult and allowed it to regenerate. He found that it regenerated less between moults the more it was mutilated. In other words Emmel finds in a general way in a nearly allied form just the opposite result to Zeleny. In 1906 Emmel found a decrease in rate of moulting as correlated with greater degree of injury and lesser rate of regeneration, again a contradictory result to that of Zeleny. With a view of testing this question the following experiment was devised. Three lots of fishes were taken designated as *A*, *B*, *C*.

In *A* the caudal fin was amputated.

In *B* the caudal and one pectoral.

In *C* the caudal and both pectorals.

The fishes were kept in separate compartments in the hatchery with running sea water but were not fed. The experiment was begun on July 30 and continued until September 4 when all were removed and measured with fine pointed registering calipers. In the following Table I. are found the results of these measurements. Not only is the length of the specimen given but the amount of regeneration and the specific regeneration. This is a term used by Zeleny. Since the specimens differ in length we must devise some means of comparing regeneration in one case with that in another. We can do this by finding the percentage regeneration in each case, *i. e.*, by dividing the amount of regeneration by the length of the specimen. We may use Zeleny's term and call it specific regeneration. In the table is also given the weight of each specimen for purposes to be described later.

Now in interpreting these results we have recourse to the statistical method. We should apply certain formulæ here to ascertain whether differences in resulting regeneration in the three

different lots are great enough to ascribe any importance to them and thus say that they are due to difference in degree of injury—or on the other hand whether after all they are not simply chance discrepancies. Workers in statistical method have deduced the law that if the mean difference of one character in any two series of forms is less than the probable errors of the difference then the

TABLE I.

Lot A.

	Length Sp.	Amt Reg. Caudal.	Sp. Reg.	Wt. Sp.
1	8.1 cm.	.55 cm.	.0676	6.27 gm.
2	8.0	.65	.0813	5.80
3	6.5	.60	.0923	3.05
4	6.35	.80	.1259	3.38
5	7.25	.65	.0896	4.20
6	9.2	.65	.0706	11.55
7	10.2	.75	.0736	16.70
8	8.25	.80	.0969	8.4
9	9.4	.70	.0744	7.5
10	7.9	.60	.0760	6.25
11	6.35	.60	.0944	3.9
12	8.4	.80	.0952	10.12
13	8.4	.60	.0714	8.85

Lot B.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	11.3	.70	.0610	.55	.0486	26.87
2	7.1	.65	.0915	.50	.0704	4.75
3	8.3	.70	.0843	.60	.0723	7.60
4	8.2	.85	.1037	.60	.0732	10.52
5	7.7	.70	.0909	.60	.0779	5.42
6	11.1	.70	.0631	.65	.0585	22.47
7	8.8	.75	.0852	.60	.0682	9.80
8	7.6	.75	.0987	.60	.0789	11.75
9	9.15	.45	.0491	.45	.0492	10.32
10	6.4	.60	.0937	.50	.0781	2.85
11	5.4	.60	.1111	.50	.0926	2.11
12	6.9	.65	.0942	.60	.0869	4.60
13	5.5	.60	.1090	.30	.0545	2.05
14	8.6	.50	.0581	.50	.0581	5.95
15	6.15	.60	.0975	.50	.0813	3.15

Lot C.

	Length Sp.	Reg Ca.	Sp. Reg. Caudal.	Reg. r. Pec.	Reg. l. Pec.	Ave. R. L Sp. Reg. Pec	Wt. Sp.
1	11.5	.55	.0478	.55	.55	.0478	23.58
2	9.15	.85	.0929	.45	.45	.0492	12.37
3	9.7	.65	.0670	.50	.45	.0489	11.1
4	7.5	.60	.0800	.60	.60	.0800	5.72
5	7.5	.50	.0666	.35	.40	.0500	4.2
6	10.0	.75	.0750	.70	.70	.0700	14.6
7	6.3	.60	.0952	.50	.55	.0833	4.1
8	6.1	.76	.1065	.40	.50	.0737	3.08
9	5.8	.80	.1379	.45	.40	.0733	2.78

two series do not differ as to that character. If, however, the mean difference is 1 + times the probable difference then it is possible that the two series do differ as to the value of that character. Also if the mean difference is 2 + times the probable difference then it is probable that the two series differ. Finally if the mean difference is 3 + times the probable difference then it is certain that the two series differ as to the value of that character. To apply the law here it is necessary to find the mean of each series, the probable error, and from these compute the mean difference and the probable error of difference.

We can arrange the results as to the caudal fin in Table I. in the form of a table.

Mean Sp. Reg.	Probable Error.	Mean Difference.	Probable Difference.
Lot A = .0860	.003106	Between A and B .0009	Between A and B .004363
Lot B = .0851	.003065	Between B and C .0005	Between B and C .005865
Lot C = .0846	.004991	Between A and C .0014	Between A and C .005876

Comparing A and B we see at once that the mean difference is actually less than the probable difference, and hence this precludes the possibility of any rational conclusion that there is any difference in regeneration in cases A and B. But we have seen that injury in case of A was less than in B. Hence we cannot conclude that the rate of regeneration is greater in the case of the less injured nor in the case of the more injured. The regeneration is the *same*.

We find the same result when we compare B and C, and also when we compare A and C. This experiment tends therefore to negative the results of both Zeleny and Emmel.

We have thus far tested the question by comparing results in these three lots as to regeneration of the caudal fin. But we can also apply the test as to regeneration of the pectorals. We can average the results of the two pectorals in Lot C.

	Mean.	Prob. Error.	Mean Diff.	Prob. Diff.
Series B	= .0694	.002353		
Series C	= .0660	.002752	.0034	.0036

In this case also the mean difference is less than the probable

difference hence the two series are the same as to the amount of regeneration in the pectoral fin — although the amount of injury was different in the two cases.

TABLE II.

Lot D.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Wt. Sp.
1	9.9 cm.	.85 cm.	.0858	16.32 gm.
2	9.2	.70	.0760	13.15
3	8.6	.70	.0818	11.35
4	7.2	.80	.1111	11.52
5	8.9	.70	.0786	6.00
6	7.2	.80	.1111	4.09
7	6.4	.65	.1015	7.10
8	7.1	.85	.1197	6.80
9	7.6	.80	.1052	4.33
10	7.9	.75	.0949	7.22
11	6.1	.70	.1147	5.85
12	7.0	.75	.1071	8.30
13	4.5	.60	.1333	1.37

Lot E.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. R. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	8.6	.70	.0818	.60	.0697	11.00
2	10.35	.55	.0531	.60	.0579	16.52
3	7.5	.60	.0800	.40	.0500	6.00
4	7.8	.70	.0897	.60	.0897	8.25
5	8.5	.70	.0823	.50	.0588	11.35
6	7.8	.70	.0897	.55	.0705	8.6
7	8.1	.50	.0617	.50	.0617	9.00
8	10.3	.70	.0679	.70	.0679	17.87
9	8.8	.70	.0795	.50	.0568	10.06
10	6.2	.60	.0967	.40	.0646	4.6
11	8.7	.70	.0804	.50	.0574	13.52
12	6.4	.80	.1250	.50	.0781	5.32
13	8.4	.70	.0833	.60	.0714	10.72
14	6.0	.75	.1250	.50	.0833	3.95
15	7.4	.60	.0811	.50	.0675	8.20
16	6.2	.70	.1129	.55	.0887	4.15

Lot F.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. L. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	7.25	.60	.0827	.60	.0827	5.67
2	7.30	.60	.0821	.35	.0410	8.03
3	7.00	.70	.1000	.50	.0714	6.47
4	6.00	.70	.1166	.60	.1000	4.07
5	6.20	.70	.1129	.40	.0646	4.63
6	6.60	.70	.1060	.60	.1060	5.28
7	6.20	.60	.0967	.50	.0806	3.98
8	6.50	.65	.1000	.40	.0631	5.32
9	6.85	.70	.1022	.60	.0876	3.91
10	6.50	.60	.0923	.45	.0692	5.13
11	6.10	.70	.1147	.50	.0819	4.27
12	6.40	.75	.1172	.35	.1171	4.63

A second experiment was started on August 2 and continued until September 5 a period of four weeks and five days practically the same as those in Table I. In this case the fish were fed regularly for it was desired to test the effect of difference in food on regeneration. In Series D the caudal alone was cut, in E the caudal and right pectoral, in F the caudal and left pectoral. Following is the table of results, Table II.

We may arrange the results as before and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot D	.1010	.002956	Between D and E .0155	.004355
Lot E	.0855	.003199	Between D and F .0018	.003977
Lot F	.1028	.002699		

Since but one pectoral fin was cut off in both E and F it is clear that we cannot compare difference in regeneration in those two lots since the degree of injury was the same. In this case we compare E with D and F with D. Here is the first exception to the uniform results obtained in series A, B and C from Table I. For on comparing E with D we find that the mean difference in regeneration is more than three times the probable difference, hence according to our formula regeneration is really greater in D than in E. At first we might think that since the two differ as to degree of injury that this must be the cause of the result — that is, that in this case at least the least injured regenerated more in the same time. But is this the case? For comparing D and F (the injury to F being the same as that to E) we see that the mean difference between the two is less than the probable difference which shows that no importance can be assigned to that mean difference and that D and F regenerated practically the same amount in the same time. The case of D and F confirms the result already found in A–B, B–C and A–C as to caudal, and in case of B–C as to pectoral. The case of D–F also shows that whatever is the cause of the discrepancy between that and D–E we cannot say that E regenerated less because it was injured more than D.

TABLE III.

Lot G.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.
1	6.1	.30	.0491
2	6.2	.25	.0403
3	7.5	.15	.0200
4	6.9	.25	.0362
5	5.9	.30	.0508
6	6.5	.30	.0461
7	7.4	.20	.0270
8	8.1	.30	.0370
9	6.5	.20	.0307
10	7.0	.20	.0285
11	7.0	.30	.0428
12	7.0	.30	.0428
13	6.8	.20	.0293

Lot H.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.
1	9.0	.20	.0222	.20	.0222
2	7.2	.40	.0555	.20	.0277
3	6.5	.25	.0384	.25	.0384
4	7.7	.20	.0259	.20	.0259
5	6.2	.30	.0484	.25	.0403
6	6.4	.20	.0312	.20	.0312
7	6.8	.30	.0441	.30	.0441
8	7.1	.30	.0422	.25	.0352
9	7.2	.20	.0277	.10	.0138
10	6.4	.30	.0468	.20	.0312

Lot I.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.	Amt. Reg. Dors.
1	7.3	.30	.0410	.20	.0274	.10
2	6.6	.30	.0454	.25	.0378	.10
3	7.2	.30	.0416	.15	.0208	.10
4	6.5	.25	.0384	.30	.0461	.15
5	7.2	.20	.0277	.20	.0277	.15
6	7.5	.20	.0266	.15	.0200	.10
7	6.4	.30	.0468	.25	.0390	.10
8	7.1	.30	.0422	.25	.0352	.15

In order to test the same question further a third experiment was set up as follows: Three lots of fishes G, H and I were operated on as follows. In G the caudal fin alone was cut off; in H the caudal and one pectoral; in I the caudal, one pectoral and the dorsal. All the fish were placed in a large aquarium with running sea water and were fed regularly. The experiment was begun on September 1 and continued until September 14, 1906. Since all the fish were kept in the same tank there is opportunity for ascertaining whether the discrepancy in case of lot E might not be due to some condition peculiar to the com-

partment in which they were kept during the five weeks they were under observation. If the results in case of G–H and I confirm the results in cases A–B, A–C, B–C, D–F, etc., then I think we are warranted in concluding that some other condition other than degree of injury was responsible for that discrepancy in case of E. The fishes in this third experiment were fed every day or so. Following is Table III. giving the measurements of these fishes.

Treating these results as before we have the following table :

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot G	.0370	.001483	Between G and H .0005	.002611
Lot H	.0375	.002149	Between H and I .0013	.003539
Lot I	.0388	.002813	Between G and I .0018	.003179

We thus see that in this case also the mean difference is not great enough to ascribe any importance to it. In each case it is less than the probable difference and hence although the series differ as to degree of injury, they do not differ as to rate of regeneration. We can also tabulate the comparative regeneration in the case of the pectorals I and H.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot H	0.0312	.001871		
Lot I	0.0320	.001985	.0008	.002727

In this case also the mean difference is less than three times the probable difference which but corroborates the above contention. We therefore find that in nine cases the result is that although the fishes differed as to degree of injury, the rate of regeneration was the same. The experiments are certainly varied enough to represent different lengths of time, etc. There is but one exception to the results and that we have shown cannot be due to difference in degree of injury.

(C) Relation of Length and Weight (i. e., Age) of Specimen to Amount Rate of Regeneration.

Zeleny ('03) observed that the rate of regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the size of the animal — that the medium sized animals have the maximum rate of regeneration. We quote from his summary, "There is a definite relation between the size (age) of the animal and the rate of regeneration. The maximum rate is exhibited by individuals of medium size. Both the larger and the smaller ones give a diminishing rate as we go away from this point." Zeleny measures size and thus age by the disk width. The three tables given in this paper furnish us with data for determining whether regeneration in *Fundulus* is greater in the smaller, medium, or larger individuals. It occurred to the writer that we ought not only to take length but weight as an indication of age. Unfortunately

Table IV.

Shortest.			Medium.			Longest.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.
6.5	.60	.0923	8.1	.55	.0676	10.2	.75	.0735
6.35	.80	.1259	8	.65	.0813	11.3	.7	.0610
7.25	.65	.0896	9.2	.65	.0706	11.1	.7	.0631
6.35	.60	.0944	8.25	.8	.0969	11.5	.55	.0478
7	.65	.0915	9.4	.7	.0744	10.0	.75	.0750
6.4	.60	.0937	8.4	.8	.0952			
5.4	.60	.1111	8.4	.6	.0714			
6.9	.65	.0942	8.3	.7	.0843			
5.5	.60	.1090	8.2	.85	.1037			
6.15	.60	.0975	7.7	.7	.0909			
6.30	.60	.0952	8.8	.75	.0852			
6.10	.65	.1065	7.6	.75	.0987			
5.8	.80	.1375	9.15	.45	.0491			
7.5	.60	.0800	8.6	.5	.0581			
7.5	.50	.0666	9.15	.85	.0929			
			9.7	.65	.0670			
			7.9	6.0	.0760			

the specimens in Table III. were not weighed and hence will not be made use of in this comparison. It has been shown by others that regeneration is a phenomenon closely related to growth. Minot has established the fact that growth is greatest in the younger forms. Hence we should expect *a priori* that regeneration should also have a greater rate in the young than in the older forms. For the purposes of this study we can take the regene-

ration in the caudal fin of all the fishes represented in Table I. This irrespective of the fact that they differ as to degree of injury, since it has just been shown that that has not affected the rate of regeneration. In Table I, we have 37 specimens — the shortest is 5.4 cm. long while the longest is 11.5 cm. long. We can divide the 37 fishes into three lots (1) the shorter, those between 5.4 cm. and 7.5 cm.; the medium between 7.6 cm. and 9.7 cm. while the longer are between 9.8 cm. and 11.9 cm. Arranging all the specimens in these three groups we have the results shown in Table IV.

We find that the average specific regeneration of the shorter is .1028, that of the medium is .0797, while that of the longer is .0638. In other words, the shortest have regenerated 10 + per cent. of their own length, the medium-sized have regenerated 7 + per cent. of their length, while the longest have regenerated 6 + per cent. of their length. Thus the result is contrary to that found by Zeleny with the brittle star-fish. But to make certain we can employ the statistical method used above and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	.0972	.002823	Between S and M =.0190	.003698
Medium	.0782	.002390	Between M and L =.0096	.003786
Longest	.0686	.002934	Between S and L =.0286	.004071

Now we find that the mean difference between S and M is not only more than three times the probable difference but nearly six times as large so that there can be no question but that the shortest have regenerated at a greater rate. On comparing S with L we find that the mean difference in regeneration is over seven times the probable difference. So that our conclusion is that the shorter have regenerated at a much greater rate than either the medium or the longer and that also the medium have regenerated at a greater rate than the longest. Now this last is confirmed when we compare M and L. In this case the mean difference is not quite three times the probable difference but more than twice the probable difference so that it is more than probable that the medium have regenerated more than the longer.

But we also have Table II. for comparison. The shorter specimens in this series are those between 4.5 cm. and 6.45 cm; the medium between 6.46 and 8.41 and the longer between 8.42 and 10.37 cm. Arranging the specimens we have Table V.

TABLE V.

Shorter.			Medium.			Longer.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.
6.4	.65	.1015	7.2	.8	.1111	9.9	.85	.0858
6.1	.7	.1147	7.2	.8	.1111	9.2	.7	.0760
4.5	.6	.1333	7.1	.85	.1197	8.6	.7	.0818
6.2	.6	.0967	7.6	.8	.1052	8.9	.7	.0786
6.4	.88	.1250	7.9	.75	.0949	8.6	.7	.0818
6	.75	.1250	7	.75	.1071	10.35	.55	.0531
6.2	.7	.1129	7.5	.6	.0800	8.5	.7	.0823
6	.7	.1166	7.8	.7	.0897	10.3	.7	.0679
6.2	.7	.1129	7.8	.7	.0897	8.8	.7	.0795
6.2	.6	.0967	8.1	.5	.0617	8.7	.7	.0804
6.1	.7	.1147	8.4	.7	.0843			
6.4	.75	.1172	7.4	.6	.0811			
			7.25	.6	.0827			
			7.3	.6	.0821			
			7	.7	.1000			
			6.6	.7	.1060			
			6.5	.65	.1000			
			6.8	.7	.1022			
			6.5	.6	.0923			

Treating the results as before, we find the average specific regeneration for the shortest is .1136, that of the medium is .0944 while that of the longest is .0763. In other words the shortest of this series of fed fishes have regenerated 11 + per cent. of their length, the medium sized have regenerated 9 + per cent. of their length while the longest have regenerated 7 + per cent. of their length.

In this case also we can apply the statistical method tabulating our results in the following form.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	=.1137	.002897	Between S and M =.0203	.003492
Medium	=.0934	.001949	Between M and L =.0170	.003066
Longest	=.0764	.002367	Between S and L =.0373	.003741

Here again it will be seen that the mean difference in regeneration between S and M is over three times the probable difference, in fact nearly six times; the mean difference between S and L shows that it is over nine times the probable difference so that there can be no doubt that the shortest specimens in Table II. regenerated at a more rapid rate than did the medium or the longest. On comparing the medium with the longest we find that the medium regenerated more rapidly than the longest as is evidenced by the fact that the mean difference is over five times the probable difference.

If we extend our hypothesis that there is a direct relation between growth and regeneration further it would be to find out whether as the fish grew older, the rate of regeneration decreased. For this is precisely the condition as regards growth — the rate of growth decreases with age. At first the decrease is rapid but then becomes very gradual. This is indicated with regard to regeneration. For on comparing regeneration of the shortest with the medium and the regeneration in the medium with the longest we discover an indication of this relation. In each of the two cases it is seen that mean difference in regeneration is greater between shortest and medium as compared with the mean difference in regeneration between the medium and longest.

It was said at the beginning of this section that in estimating age we ought to consider both length and weight. So I have placed opposite the length, etc., of each specimen its weight. *A priori*, the youngest ought to be the shortest and lightest, etc. This is the case here, for the average weight of the shortest is 3.38 gm.; the average weight of the medium length is 8.96 gm., while that of the longest is 20.85 gm. So that in Table IV. our three classes represent the youngest, the oldest and those midway between the others in age. In the same way for Table V. the average weight of the shortest is 4.48 gm.; that of the longest is 12.71 gm. while that of the medium length is 7.52 gm. In this case also our three series in Table V. represent the youngest, the oldest and the medium aged fishes of that lot. We conclude therefore that the rate of regeneration in *Fundulus h.* is greatest in the youngest fishes, less in the older, and that there is a slight indication that the rate of regeneration decreases with age.

(D) Relation of Regeneration to Food.

It will be remembered that fishes in Series A, B and C were not fed while those in Series D, E and F were fed. Otherwise as far as known they were kept under identical conditions and for the same length of time. It was desired to compare regeneration in the two series with each other to ascertain whether regeneration is at a greater rate in the fed fishes than in the not-fed. Unfortunately we cannot make use of Series E because as before said some factor has entered there and caused a result which I do not believe to be normal. On the other hand I do regard Series D and F as normal. Therefore we must compare regeneration in D with that in A.

I. Caudal.	Mean Reg.	Prob. Error.	Mean Diff	Prob. Diff.
Lot D (fed)	=.1010	.003106		
Lot A (not fed)	=.0860	.002958	.0150	.004249

In this case the mean difference in regeneration is more than three times the probable difference. In other words the fishes in Lot D which were fed have regenerated more in the same time than the fishes in Lot A which were not fed.

II. Caudal.	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot F (fed)	=.1028	.002659		
Lot B (not fed)	=.0851	.003065	.0177	.004084

In this case also the mean difference in regeneration between F and B is more than three times the probable difference. Or the fishes in Lot F which were fed have regenerated more than the fishes in Lot B which were not fed.

We may compare the average specific regeneration of the caudal in the case of the fishes that were fed with that in the fishes that were not fed in the following way also.

Average Sp. Reg. in caudal of Lots D + F (fed)............................ =0.10100
Average Sp. Reg. in caudal of Lots A + B + C (not fed)................. =0.08565
Difference... =0.01535

The caudal fin in fishes that were fed regenerated 18 per cent. more than that in fishes not fed.

Again,

Average Sp. Reg. in pectoral fin in Lot F (fed)................................ =0.0866
Average Sp. Reg. in pectoral fin in Lot B + C (not fed)..................... =0.0677
Difference... =0.0189

that is, the pectoral in the fed fishes regenerated 24 per cent. more than that in the unfed.

(E) Regeneration in Fins used most Compared with that in Less used Fins.

Adaptation in Regeneration. — Osburn ('06) experimenting on the fins in *Fundulus* finds that the pectorals are not used as vigorously as the caudal, and the dorsal not so much as the pectorals. Broussonet (1786) stated that the most useful fins regenerate more rapidly than those less useful. Morrill ('06) says that the "caudal fin though obviously the most important does not regenerate perceptibly faster than the others." Although the data are insufficient to settle this question which is to a great extent a matter of interpretation yet the following table is suggestive. Since it has been shown that degree of injury has made no difference in the rate of regeneration in *Fundulus h.*, it is therefore possible to group all the specimens represented in Table I. thus,

Average Sp. Reg. of caudal fin in Lots A + B + C	= 0.08565
Average Sp. Reg. of pectoral fin in Lots B + C	= 0.06770
Difference	= 0.01795

which means that the caudal fin of specimens in Lots A + B + C regenerated 26 per cent. more than the pectoral fin in Lots B + C in the same time and under same conditions.

In the same way from Table II., we find

Average Sp. Reg. of caudal fin in Lots D + F	= 0.01016
Average Sp. Reg. of pectoral fin in Lot F	= 0.00846
Difference	= 0.00170

or in other words the caudal regenerated 20 per cent. more than the pectoral.

Again in Table III. the

Average Sp. Reg. in caudal in Lots G + H + I	= 0.0386
Average Sp. Reg. in pectoral in Lots H + I	= 0.0313
Difference	= 0.0073

or the caudal in this case has regenerated 20 per cent. more than the pectoral. Thus in all three cases the result is the same. It should be remembered that specific regeneration is the relation of amount regenerated to the length of the specimen. And hence we have a right to compare the specific regenerations of caudal with pectoral. It may also be pointed out here that the dorsal

fin regenerates very slowly as compared with the other two as may be seen in Table III.

General Conclusions.

1. That low temperatures inhibit regeneration in caudal fin of *Fundulus heteroclitus*.

2. That the rate of regeneration bears no relation to the degree of injury to the caudal and pectoral fins.

3. That regeneration is greater in the younger than in the medium and older fishes which is in line with the theory that regeneration is a growth phenomenon.

4. That regeneration is greater in the fish that have been fed as compared with that in the fishes not fed.

5. That there *is* an indication that the fins used or needed most (*i. e.*, caudal) regenerate more rapidly than the less needed fin (pectoral), or in other words there is an indication of adaptation in regeneration.

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